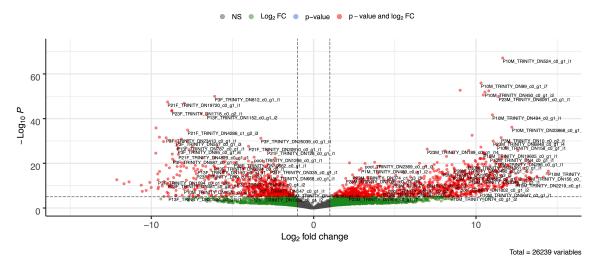
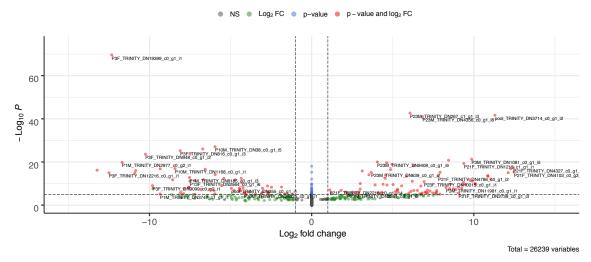
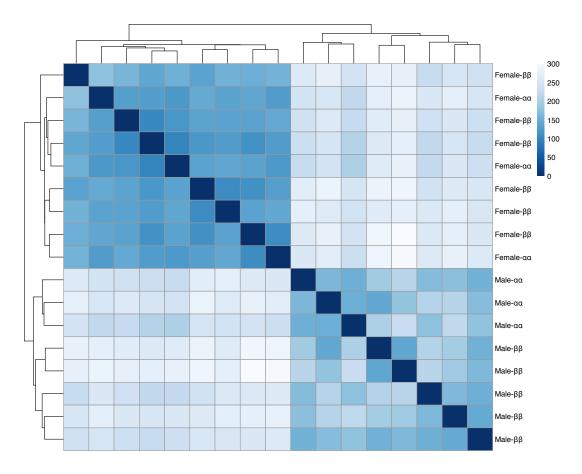
Supplemental Figures



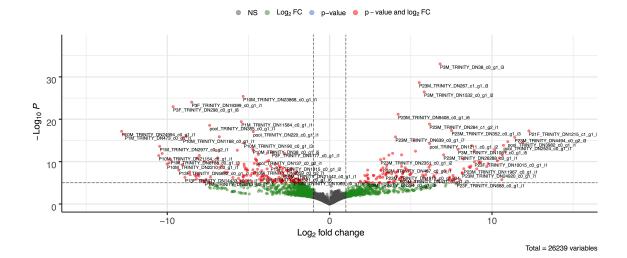
<u>Supplemental Figure 1</u> - Differential expression by sex in adults. Positive values indicate higher expression in males and negative values indicate higher expression in females. Each dot represents a single gene and is colored to indicate significance. Grey - not significant, Green - significant by \log_2 fold change (>2), Blue - significant by p-value (<0.001), Red - significant by \log_2 fold change and p-value. Figure made with EnhancedVolcano implemented in R [1].



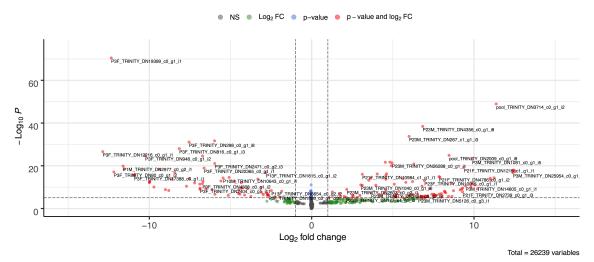
Supplemental Figure 2 - Differential expression by karyotype in adults. Positive values indicate higher expression in $\beta\beta$ and negative values indicate higher expression in $\alpha\alpha$. Each dot represents a single gene and is colored to indicate significance. Grey - not significant, Green - significant by \log_2 fold change (>2), Blue - significant by p-value (<0.001), Red - significant by \log_2 fold change and p-value. Figure made with EnhancedVolcano implemented in R [1].



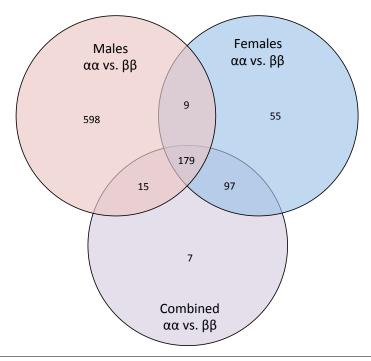
<u>Supplemental Figure 3</u> - Heatmap of Euclidian sample distances in adults. Samples are labeled with sex and karyotype. Color indicates distance, with darker blues being more similar. Figure made with pheatmap implemented in R [2].



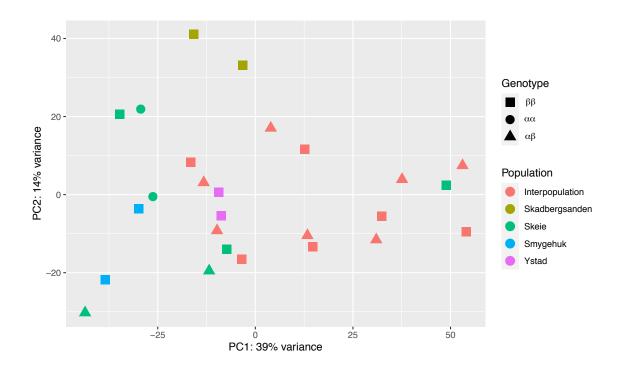
Supplemental Figure 4 - Differential expression by karyotype in adult males. Positive values indicate higher expression in $\beta\beta$ and negative values indicate higher expression in $\alpha\alpha$. Each dot represents a single gene and is colored to indicate significance. Grey - not significant, Green - significant by \log_2 fold change (>2), Blue - significant by p-value (<0.001), Red - significant by \log_2 fold change and p-value. Figure made with EnhancedVolcano implemented in R [1].



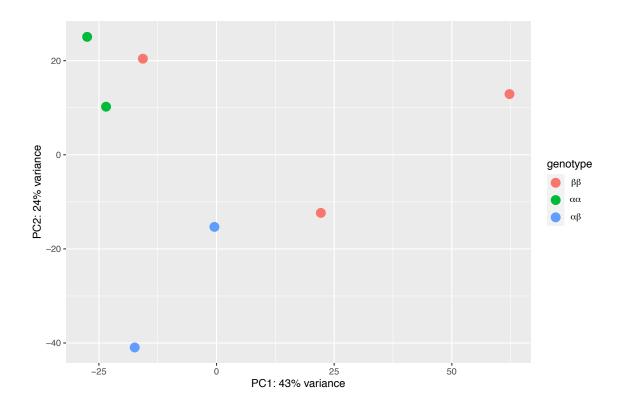
Supplemental Figure 5 - Differential expression by karyotype in adult females. Positive values indicate higher expression in $\beta\beta$ and negative values indicate higher expression in $\alpha\alpha$. Each dot represents a single gene and is colored to indicate significance. Grey - not significant, Green - significant by \log_2 fold change (>2), Blue - significant by p-value (<0.001), Red - significant by \log_2 fold change and p-value. Figure made with EnhancedVolcano implemented in R [1].



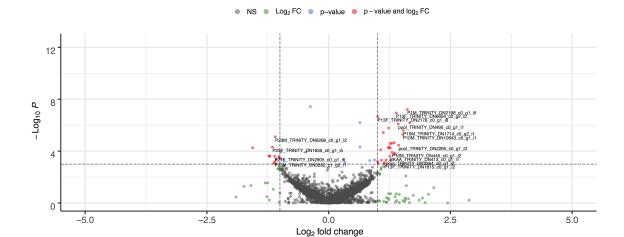
Supplemental Figure 6 - Overlap between differential expression test sets. Pictured is the number of significantly differentially expressed transcripts that overlapped between our 3 test sets: male $\alpha\alpha$ vs. $\beta\beta$, female $\alpha\alpha$ vs. $\beta\beta$, combined (i.e. both sexes) $\alpha\alpha$ vs. $\beta\beta$.



Supplemental Figure 7 - Expression variation by population in larvae. Points are colored by population (Interpopulation - red, Skadbergsanden - mustard, Skeie - green, Smygehuk - blue, and Ystad-purple) and shaped according to karyotype ($\alpha\alpha$ -triangle, $\alpha\beta$ -square, $\beta\beta$ -circle). Interpopulation refers to larvae that were generated by interpopulation crosses, see supplemental table 1 for cross types.

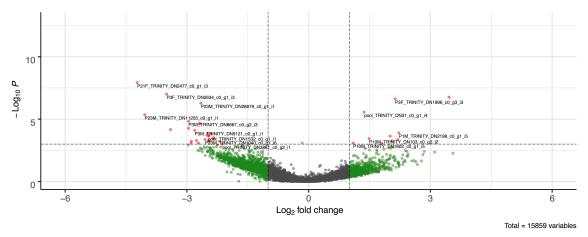


Supplemental Figure 8 - Principal component analysis (PCA) of expression variation in larvae from Skeie. Points are colored by karyotype ($\alpha\alpha$ - green, $\alpha\beta$ -blue, $\beta\beta$ -red).

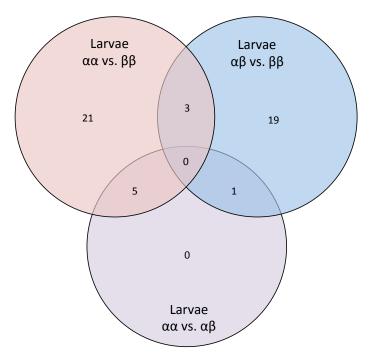


Supplemental Figure 9 - Differential expression by genotype in larvae. Positive values indicate higher expression in $\alpha\beta$ and negative values indicate higher expression in $\beta\beta$. Each dot represents a single gene and is colored to indicate significance. Grey - not significant, Green - significant by \log_2 fold change (>2), Blue - significant by p-value (<0.001), Red - significant by \log_2 fold change and p-value. Figure made with EnhancedVolcano implemented in R [1].

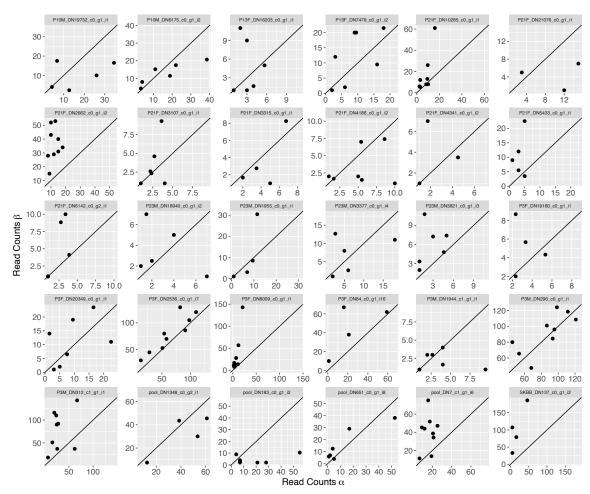
Total = 15859 variables



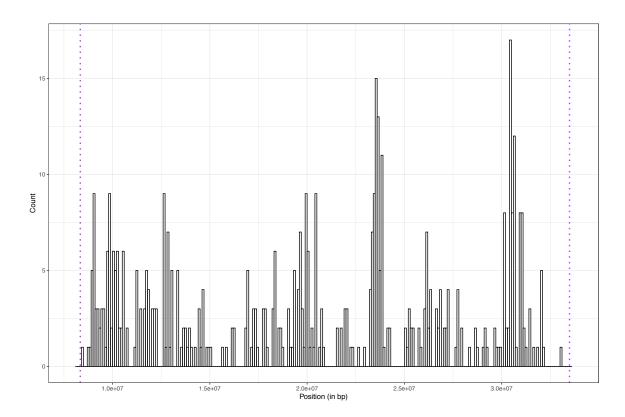
Supplemental Figure 10 - Differential expression by genotype in larvae. Positive values indicate higher expression in $\alpha\alpha$ and negative values indicate higher expression in $\beta\beta$. Each dot represents a single gene and is colored to indicate significance. Grey - not significant, Green - significant by \log_2 fold change (>2), Blue - significant by p-value (<0.001), Red - significant by \log_2 fold change and p-value. Figure made with EnhancedVolcano implemented in R [1].



Supplemental Figure 11 - Overlap between differential larval expression test sets. Pictured is the number of significantly differentially expressed transcripts that overlapped between our 3 test sets: $\alpha\alpha$ vs. $\beta\beta$, $\alpha\beta$ vs. $\beta\beta$, and $\alpha\alpha$ vs. $\alpha\beta$.



 $\frac{Supplemental\ Figure\ 12}{Each\ plot\ is\ for\ a\ single\ transcript}\ where\ each\ dot\ represents\ a\ single\ individual\ averaged\ over\ all\ SNPs\ in\ that\ transcript.\ A\ 1:1\ line\ is\ provided\ for\ context.$



Supplemental Figure S13 - Positions of all differentially expressed genes located within Cf-Inv(1). Shown are positions of genes differentially expressed between $\alpha\alpha$ vs. $\beta\beta$ in males, females, or larvae. Genes are binned over 100 kb. Breakpoints of Cf-Inv(1) are indicated with dashed magenta lines.

- 1. Blighe K, Rana S, Lewis M. EnhancedVolcano: Publication-ready volcano plots with enhanced colouring and labeling. R package version. 2019;1(0).
- 2. Kolde R, Kolde MR. Package 'pheatmap'. 2015.